Acid-Catalysed Dehydration of Dehydroretinol

By P. G. NAYAR

Department of Chemistry, N.S.S. College, Pandalam, Kerala, India

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1. Treatment of dehydroretinol with hydrogen chloride in light petroleum or toluene-*p*-sulphonic acid in benzene produces a substance with u.v.-absorption maxima at 284, 296, 310, 350, 368, 388, 408 and 433nm. and antimony trichloride colour maximum at 620nm. This compound is tentatively named compound 433 after its absorption maximum at 433nm. 2. Treatment of dehydroretinol with ethanolic hydrogen chloride produces, besides 3-ethoxyanhydroretinol, some compound 433 also. 3. Compound 433 is present in several freshwater-fish liver oils. 4. The i.r. spectrum of compound 433 shows only a band attributable to a conjugated ethylenic system. 5. The course of changes that lead to the formation of compound 433 is discussed.

Edisbury, Gillam, Heilbron & Morton (1932) first observed that treatment of a retinol concentrate with ethanolic hydrogen chloride gives a hydrocarbon with sharp u.v.-absorption bands at 350, 370 and 392nm. Shantz, Cawley & Embree (1943) crystallized this product from light petroleum as clusters of orange prisms showing u.v.-absorption

maxima at 351, 371 and 390nm. and antimony trichloride colour maximum at 620nm., and named it anhydrovitamin A₁ (anhydroretinol). The structure proposed by Meunier, Dulou & Vinet (1943) (I), which would readily explain its formation from retinol by acid-catalysed dehydration, has been accepted for this substance. Small quantities of anhydroretinol have been found to be present in several fish liver oils.

By an analogous procedure Shantz (1948) prepared anhydrovitamin A₂ from dehydroretinol. The u.v.-absorption spectrum of this compound was identical with that of anhydroretinol, but in the antimony trichloride test it showed maximum absorption at 693nm. By analogy with the structure of anhydroretinol, anhydrovitamin A₂ was believed to be a hydrocarbon with a 2-dehydro-anhydroretinol structure. However, such a structure would not explain its u.v.-absorption spectrum or its stronger adsorbability on alumina, which facilitated its separation from anhydroretinol. The problem was settled by Henbest, Jones, Owen & Thaller (1955), who proved by i.r. studies that anhydrovitamin A₂ is 3-ethoxyanhydroretinol (II), the ethoxy group having been derived from the ethanol in which the dehydration is carried out. Additional evidence for this was given by Balasundaram, Bamji, Cama, Sundaresan & Varma (1958), who were unable to detect any anhydrovitamin A₂ on treatment of dehydroretinol with toluene-*p*-sulphonic acid, which gives anhydroretinol with retinol.

Another kind of anhydrovitamin A₂ found in freshwater-fish liver oils and called 'naturally occurring anhydrovitamin A₂' was reported by Balasundaram *et al.* (1958) and Barua & Nayar (1963, 1966). Its u.v.-absorption spectrum and

(I)

Anhydroretinol

(II)

3-Ethoxyanhydroretinol
 (anhydrovitamin A₂)

(III)

3-Hydroxyanhydroretinol
 ('naturally occurring anhydrovitamin A₂')

antimony trichloride colour maximum are identical with those of anhydrovitamin A₂, but it is much more strongly adsorbed on alumina. This has been shown to be 3-hydroxyanhydroretinol (Barua & Nayar, 1966) (III).

The present paper reports a reinvestigation of the action of hydrogen chloride and toluene-*p*-sulphonic acid on dehydroretinol in solvents such as light petroleum, benzene and ethanol. In all cases the main product is a hitherto unreported hydrocarbon with a rather unusual spectrum. This substance is tentatively named compound 433 after its absorption maximum at 433 nm. Further, this compound, to which a reference was made by Barua & Nayar (1966) as a 'hitherto unidentified compound, probably an unsaturated hydrocarbon' is shown to be present in several freshwater-fish liver oils.

EXPERIMENTAL

Apparatus and reagents. The u.v. spectra were recorded on a Beckman DK-2 spectrophotometer. The absorption spectra of the colours produced by the various compounds with SbCl₃ reagent were also recorded on the same instrument. The i.r. spectrum was recorded on a Perkin-Elmer instrument.

The reagents were all purified by standard procedures before use (Barua & Nayar, 1966). The alumina used for chromatography was obtained from Sarabhai-Merck Ltd., Baroda, India.

Preparation of dehydroretinol. A light-petroleum solution of *Bagarius bagarius* liver oil was chromatographed on alumina [5% (v/w) of water], and the dehydroretinol esters fraction, which could be identified by its formation of a green colour with SbCl₃ reagent and its u.v.-absorption spectrum, was eluted with light petroleum (b.p. 40–60°). It was chromatographed on deactivated alumina until $E_{1\text{cm}}^{1\%}$ at 351 nm. was 648.3, and was then saponified with ethanolic KOH. The free dehydroretinol was extracted with diethyl ether, taken up in light petroleum and chromatographed on alumina deactivated with 10% (v/w) of water. Dehydroretinol was eluted with light petroleum containing 10–15% (v/v) of diethyl ether. The chromatography was repeated until $E_{1\text{cm}}^{1\%}$ at 351 nm. rose to 1371.

Action of HCl on dehydroretinol. Dehydroretinol (20 mg.) was kept in contact with a 15.3 mm solution of pure dry HCl gas in light petroleum (b.p. 40–60°) (50 ml.) for 15 min. at 25° in the dark. It was then shaken with Na₂CO₃ (200 mg.), decanted off, concentrated and chromatographed on alumina [25 g., 3% (v/w) of water]. A yellow zone, eluted with light petroleum, showed the following spectral properties: λ_{max} 284 ($E_{1\text{cm}}^{1\%}$ 619), 296 ($E_{1\text{cm}}^{1\%}$ 715), 310 ($E_{1\text{cm}}^{1\%}$ 901), 350 ($E_{1\text{cm}}^{1\%}$ 1243), 368 ($E_{1\text{cm}}^{1\%}$ 1491), 388 ($E_{1\text{cm}}^{1\%}$ 1196), 408 ($E_{1\text{cm}}^{1\%}$ 1087) and 433 nm. ($E_{1\text{cm}}^{1\%}$ 948), and SbCl₃ colour λ_{max} 620 nm. ($E_{1\text{cm}}^{1\%}$ 1842).

An attempt to follow the reaction spectrophotometrically provided the results presented in Table 1. The acid reaction mixture always showed the spectral characteristics of 3-ethoxyanhydroretinol and conversion into compound 433 occurred only on neutralization of the acid.

Action of toluene-*p*-sulphonic acid on dehydroretinol. A 0.76 mm solution of toluene-*p*-sulphonic acid in anhydrous

Table 1. *Spectral results on the reaction of dehydroretinol with a solution of hydrochloric acid in light petroleum*

Solution	λ_{max} . (nm.)	SbCl ₃ colour λ_{max} . (nm.)
1. Original dehydroretinol	287, 351	693
2. After adding HCl solution	350, 368, 390	693
3. 5 min. after addition	350, 368, 390	693
4. 10 min. after addition	350, 368, 390	693
5. After neutralization with Na ₂ CO ₃	284, 296, 310, 350, 368, 388, 408, 433	620
6. After chromatography	284, 296, 310, 350, 368, 388, 408, 433	620

benzene (5 ml.) was added to dehydroretinol (20 mg.) and after 1 min. the solution was shaken with Na₂CO₃ (20 mg.) and filtered. The filtrate was evaporated to dryness *in vacuo*, and the residue dissolved in light petroleum was chromatographed on alumina. A substance having the spectral characteristics described earlier was eluted with light petroleum.

The same results could be obtained by adding a few crystals of toluene-*p*-sulphonic acid to a solution of dehydroretinol in light petroleum, shaking and filtering.

Action of HCl in ethanol on dehydroretinol. A 67 mm solution of dry HCl gas in ethanol (40 ml.) was added to dehydroretinol (40 mg.) and the mixture was kept in the dark for 20 min. Saturated Na₂CO₃ solution (5 ml.) was then added and after dilution with water the solution was extracted with light petroleum. The extract was dried with Na₂SO₄, concentrated *in vacuo* and chromatographed on alumina [5% (v/w) of water]. Two fractions were eluted with light petroleum. The faster-moving fraction showed all the spectral characteristics of compound 433 described earlier. The other fraction was identified as 3-ethoxyanhydroretinol from its following spectral properties: λ_{max} 350 ($E_{1\text{cm}}^{1\%}$ 1986), 368 ($E_{1\text{cm}}^{1\%}$ 2692) and 390 nm. ($E_{1\text{cm}}^{1\%}$ 2176), and SbCl₃ colour λ_{max} 693 nm. ($E_{1\text{cm}}^{1\%}$ 3895).

Isolation of compound 433 from liver oils. A solution of *Bagarius bagarius* liver oil (2 g., $E_{1\text{cm}}^{1\%}$ at 351 nm. 150) in light petroleum was chromatographed on deactivated alumina [150 g., 5% (v/w) of water]. The chromatogram was initially developed with light petroleum. The eluate was collected in fractions (1 ml.) and these were examined spectrophotometrically. The first constituent to flow out of the column was identified as anhydroretinol from its u.v. spectrum with maxima at 350, 369 and 390 nm., SbCl₃ colour with maximum at 620 nm., greenish-yellow fluorescence in u.v. light and its behaviour on mixed chromatography with pure anhydroretinol.

Closely following this, compound 433 was eluted. On the column it could be distinguished from anhydroretinol by its pink fluorescence under u.v. light. This material constituted about 0.1% (by weight) of the original oil. After further purification by careful chromatography on alumina [1% (v/w) of water] the following spectral results (solvent light petroleum) were obtained: λ_{max} 284 ($E_{1\text{cm}}^{1\%}$ 568), 296 ($E_{1\text{cm}}^{1\%}$ 628), 310 ($E_{1\text{cm}}^{1\%}$ 961), 350 ($E_{1\text{cm}}^{1\%}$ 1216), 368 ($E_{1\text{cm}}^{1\%}$

1412), 388 ($E_{1\text{cm}}^{1\%}$, 1137), 408 ($E_{1\text{cm}}^{1\%}$, 1020) and 433 nm. ($E_{1\text{cm}}^{1\%}$, 863), and SbCl_3 colour λ_{max} , 620 nm. ($E_{1\text{cm}}^{1\%}$, 1792).

Mixed chromatography of natural and synthetic compound 433. A light-petroleum solution (3 ml.) containing natural compound 433 (10 mg.) obtained from *Bagarius bagarius* liver oil and synthetic compound 433 (10 mg.) from dehydroretinol was chromatographed on alumina [25 g., 1% (v/w) of water]. Only a single yellow zone was present on the column and under u.v. light only one pink band was seen. It was eluted with light petroleum and the absorption spectrum was examined. The spectrum was unaltered, indicating the identity of the two substances.

U.v.-absorption spectra of compound 433 in light petroleum (—), ethanol (---) and chloroform (-.-.-).

The mixed chromatography was repeated on a thin layer of alumina, on a magnesia-Celite mixture and on Vaseline-coated filter paper (reversed phase). All the experiments proved the complete identity of the synthetic and natural products. Moreover, the u.v. spectrum of the compound remained unaltered, indicating that it was a single substance and not a mixture as the rather unusual u.v. spectrum might suggest.

Irradiation of compound 433. The u.v. spectrum of a pure specimen of compound 433 in light petroleum was recorded. The solution contained in the spectrophotometer cell itself was then exposed to an incandescent filament lamp (100 w) kept at a distance of 2 ft. from the cell. The absorption spectrum of the solution was recorded after intervals of 2 min., 5 min., 10 min., etc. The spectra indicated only progressive degeneration of the compound. No isosbestic point was obtained, showing that compound 433 was not a mixture of two photochemically interconvertible substances.

Absorption spectra of compound 433 in other solvents. Fig. 1 shows the u.v. spectra of compound 433 in light petroleum, ethanol and chloroform at identical concentrations.

Infrared spectrum of compound 433. The i.r. spectrum of compound 433 was determined. Except for a peak at 1650cm^{-1} , the spectrum showed no absorption bands characteristic of any functional groups.

DISCUSSION

The foregoing experiments show that compound 433 isolated from *Bagarius bagarius* and other liver oils is formed from dehydroretinol by application of the same dehydration methods as are used for the preparation of anhydroretinol.

The very weak adsorbability of compound 433 on alumina points to the fact that it is a hydrocarbon. During chromatography of freshwater-fish liver oils on alumina, the position of compound 433 is just above anhydroretinol and below β -carotene. The

Scheme 1.

rather complicated u.v. spectrum of compound 433 gives the impression that it is not a single substance, but the available evidence shows that it is not a mixture. For example, irradiation of compound 433 in light petroleum brings about only decomposition, and no isosbestic point is obtained in the spectrum of the solution. Repeated chromatography by various methods also did not reveal any separation into components. Except for a peak at 1650cm^{-1} , which must be due to a conjugated ethylenic system, the i.r. spectrum of compound 433 shows no absorption bands characteristic of any group.

A consideration of the mechanism of formation of compound 433 would be premature, as it has not been possible to make any suggestion about its structure. However, the mechanism must be similar, at least in the initial stages, to the now generally accepted mechanism of formation of 3-ethoxy-anhydroretinol. The dehydroretinol molecule (Scheme 1, IV) first loses its hydroxy group and generates the carbonium ion (V), which undergoes allylic rearrangement to structure (VI). Elimination of hydrogen from C-2 with the formation of a double bond is considered impossible, as the molecule so formed would be highly strained (Henbest *et al.* 1955). The carbonium ion (VI) therefore reacts with the solvent ethanol and forms 3-ethoxyanhydroretinol. The results presented in

Table 1 show that the carbonium ion (VI) is formed on treatment of dehydroretinol with hydrogen chloride in light petroleum also. However, since light petroleum cannot supply a nucleophilic group like $\text{C}_2\text{H}_5\text{O}^-$, neutralization of the reaction mixture with sodium carbonate brings about some other change leading to the formation of compound 433.

Compound 433 has also been found to be present in other dehydroretinol-rich freshwater-fish liver oils, e.g. those of *Wallago attu*, *Silonia silondia*, *Pangasius pangasius*, etc. (Barua & Nayar, 1964), and it is surprising that there has been no previous reference to its occurrence.

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